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Specific Aims

The main objective of these studies was to study the CNS activity of these plant fractions using specific radio ligand binding assays to identify their selectivity profiles as well as to determine their functional activities as either a full or partial agonist or antagonist.

Materials

The following table gives a list of the samples provided to us thus far.

Table 1: ICBG PLANT SAMPLES

In House Sample#	SU-Lab Number	Plant Part	Wt given (mg)
ICBG 1	SU1904	Whole Plnt	25
ICBG 2	SU1905	Sd Pulp	25
ICBG 3	SU1906	Pn polar	25
ICBG 4	SU1907	Lf/Stem	25
ICBG 5	SU1908	Lf/stem	25
ICBG 6	SU1909	Stbk	25
ICBG 7	SU1910	Stbk	25
ICBG 8	SU1911	Stbk	25
ICBG 9	SU1912	Whole Plnt	25
ICBG 10	SU1913	Whole Plnt	25
ICBG 11	SU1914	Ft pulp	25

Methods

Preparation of Extracts: Stocks of the solid extracts were prepared by weighing 4 mg of sample and diluting to a total volume of 5 ml with 50% ethanol solvent for a final concentration of 0.8 mg/ml.

Opioid Recetor(kappa-1) Ligand Binding[a]: Two-point binding screens using [^{3}H]U69593 were performed as per metheds within the grant and previously (Izenwasser et al,1999). Briefly, mokey insular cortex was homogenized in ice-cold buffer (1:10 w/w, 50 mM Tris, 176 µM $MnCl_2$, 0.1% BSA pH 7.4), centrifuged at 32,000 x g for 10 minutes, and the supernatant was discarded. The pellet was resuspended (1:10, w/w) with buffer, and 100 µl was added to each tube for a final concentration of 10.0 mg/ml (original tissue wet weight), in a final assay tube volume of 1.0 ml. Screens were performed by incubating 2 concentrations of extract (0.8 and 80

µg/tube) in the presence 1.0 nM of radioligand for 1 hr at 25°C, to determine their inhibitory potency (% inhibition). Nonspecific binding was determined by binding in the presence of 10 µM naloxone. Incubations were terminated by vacuum filtration through glass fiber filters (Whatman 934-AH) presoaked in 1% polyethylenimine (PEI) followed by washing with ice-cold buffer (2 x 5 ml).

Norepinephrine Transporter(NET) Ligand Binding[b]*:* Two-point binding screens using [^{3}H]Nisoxetine were performed as per metheds within the grant and previously (Tejani-Butt et al,1990). Briefly, mokey hypothulamus was homogenized in ice-cold buffer (1:10 w/w, 50 mM Tris, 300 mM NaCl, 5 mM KCl pH 7.4), centrifuged at 32,000 x g for 10 minutes, and the supernatant was discarded. The pellet was resuspended (1:10, w/w) with buffer, and 100 µl was added to each tube for a final concentration of 10.0 mg/ml (original tissue wet weight), in a final assay tube volume of 1.0 ml. Screens were performed by incubating 2 concentrations of extract (0.8 and 80 µg/tube) in the presence 3.0 nM of radioligand for 4 hr at 4°C, to determine their inhibitory potency (% inhibition). Nonspecific binding was determined by binding in the presence of 10 µM Mazindol. Incubations were terminated by adding 4ml of ice-cold buffer and vacuum filtration through glass fiber filters (Whatman 934-AH) presoaked in 0.5% polyethylenimine (PEI) followed by washing with ice-cold buffer (2 x 4 ml).

Data Analysis: Data are analyzed using the non-linear regression algorithms found in EBDA/LIGAND™ and GraphPad Prism™ computer software programs.

Results.

Table 2: Plant Extract Activity (% inhibition) on DAT, SERT, Mµ, Kappa-1 & NET

In House Sample#	*DAT* ([^{3}H]*WIN35,428*) @ 0.8 µg/tube	@ 80 µg/tube	*SERT* ([^{125}I]RTI-55) @ 0.8 µg/tube	@ 80 µg/tube	*Mµ* ([^{3}H]DAMGO) @ 0.8 µg/tube	@ 80 µg/tube	*Kappa-1* ([^{3}H]U69593) @ 0.8 µg/tube	@ 80 µg/tube	*Kappa-1* ([^{3}H]U69593) IC50 Ug/ml	nH
ICBG 1	--	58.7		42.0	1.8	60.4	--	84.0	37.4	1.21
ICBG 2	--	46.3	--	90.1	16.7	60.2	--	91.1	15.9	0.93
ICBG 3	--	18.8	--	46.7	31.8	39.4	--	78.8	51.6	1.32
ICBG 4	--	77.7	--	--	33.0	41.0	--	82.1	72.6	1.25
ICBG 5	--	22.9	--	--	--	33.3	--	--	N/A	
ICBG 6	--	27.2	--	20.5	--	33.4	--	51.6	68.6	1.49
ICBG 7	--	--	--	--	--	29.6	--	9.9	N/A	
ICBG 8	--	50.6	--	31.0	10.3	29.2	--	64.0	44.9	1.04
ICBG 9	--	82.0	--	--	--	32.8	--	64.0	81.8	1.13
ICBG 10	--	74.9	3.9	76.1	28.6	32.9	--	74.5	47.8	1.11
ICBG 11	--	--	--	87.2	32.4	47.1	--	41.1	N/A	

-- denotes no inhibitory activity

As can be seen in Table 2, a number of extracts inhibited greater than 50% of the binding at DAT, SERT and Mµ. Therefore, full characterization of these compounds will be conducted.

Future Aims

Future studies will be to further characterize those extracts found to be active with full competition curves. In addition we plan to expand the screens with other ligands specific to other neuroreceptors and neurotransporters, and adding new compounds as made available.

References

[a]Sari Izenwasser, Julie K. Staley, Stephanie Cohn, and Deborah C. Mash. Characterization of Kappa-Opioid Receptor Binding in Human Insular Cortex. *Life Sciences 1999;*. **65**(9):857-862.

[b]Shanaz M, Tejani-Butt, David J, Brunswick and Alan Frazer. [^{3}H]Nisoxetine: a new radioligand for norepinephrine uptake sites in brain. *European Journal of Pharmacology 1990*; **191**:239-243.